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Short Communication

OCCURRENCE OF VEROTOXIC E. COLI IN FAECES AND MILK OF CATTLE

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SUMMARY

Verotoxic *E. coli,* an emerging human pathogen, is known to be transmitted from animals to man through foods contaminated by cattle faeces. In the present study, 12 out of 200 faeces samples of healthy and diarrheic cattle, and one of 100 milk samples were found positive for verotoxic *E. coli*. The isolation of these *E. coli* from healthy cattle (11/200) confirms the reservoir status of cattle for these *E. coli*. Among O:10, O:22, O:26, O:111 and O:136 serotypes isolated from these samples, O:111 was predominant which is known for causing hemorrhagic colitis and haemolytic uremic syndrome in man. Isolation of verotoxic *E. coli* from diarrheic and healthy animals living in close contact of human beings suggests its public health significance.

Key words: Verotoxic, *Escherichia coli*, milk, cattle

Among the diarrhea causing *Escherichia coli*, enterohaemorrhagic *E. coli* (EHEC) have emerged as important food-borne pathogens and are responsible for hemorrhagic colitis (HC), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in human beings (Hiruta *et al.*, 2001). They are known for production of verotoxins and hence termed as verotoxic *E. coli* (VTEC). Apparently healthy animals especially dairy cattle have been known to act as reservoir of these types of *E. coli* (Montenegro *et al.,* 1990). They are transmitted from animals to human beings through milk, meat and their products specially ground beef (Chapman and Higgins, 1993). A number of outbreaks of HC and HUS have been reported in the United Kingdom, Canada and United States by *E. coli* O157: H7, and in Australia and Germany by non-O157 serotypes like O: 26, O: 55, and O: 111 (Riley *et al.*, 1983, Wilson *et al.*, 1997, Hiruta *et al.*, 2001). In India, significance of EHEC has been recognized recently and *E. coli* O: 157 was isolated in India about a decade ago from buffalo meat (Singh *et al.*, 1996), but other VTEC have been isolated from patients of bloody diarrhea and haemorrhagic enteritis (Kapoor *et al.*, 1995), from healthy cattle (Pal *et al.*, 1999) and foods of animal origin (Banerjee *et al.*, 2001). Keeping in view the emergence of VTEC as human pathogen and scanty work done in India, the present study was undertaken to study the status of dairy cattle of Hisar and surrounding area as a principle reservoir of verotoxic *E. coli.*

Three hundred samples comprising 200 faecal matters (50 each of healthy cows and cow calves, 100 diarrheic calves) and 100 raw cow milk were collected aseptically from different organized and unorganized cattle farms and milk vendors of Hisar and its surrounding area. Preliminary isolation of *E. coli* from faeces was done as per the method of Wallace and Jones (1996) using modified tryptone soya broth (mTSB) as enrichment medium and cefixime-tellurite sorbitol MacConkey agar (CT-SMAC) as selective medium while isolation from milk samples was done as per the method of Adesiyun *et al.* (1997) using Eosine Methylene Blue (EMB) agar. Presumptive isolates of *E. coli* were identified by the methods described by Edwards and Ewing (1972) and isolates were got serogrouped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh, India. The cytotoxicity of representative isolates of *E. coli* was evaluated on Vero cell line as per the method described by Konowalchuk *et al.* (1977).

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Among 200 samples of faeces of adult cattle and calves and 100 of raw milk, 12 (6.0%) and 1 (1%) samples were positive for verotoxic *E. coli*, respectively. A total of 18 isolates of VTEC were obtained from these 13 positive samples, of which 13 isolates were from 9 samples of healthy adult cattle, 3 from 2 of healthy calves and one each from diarrheic calf and cow milk. Thirteen isolates from healthy cattle were categorized into four serogroups viz. O: 10, O: 26, O: 111, O: 136, three isolates from healthy calves were typed as O: 136 and O: 111, one isolate each from diarrheic calf and cow milk belonged to O: 22 and O: 28 serogroups, respectively. Predominant serogroup of VTEC prevalent in this area was O: 111 as 8 of 18 (44.0 %) isolates belonged to this group. All the 8 isolates of O: 111 and 3 of O: 26 were found verocytotoxic. None of the isolates from positive samples of faeces and milk, however, belonged to O: 157 serogroup, as they did not produce yellow colonies on CT- SMAC and all fermented sorbitol. In Australia and Germany, non-O: 157 VTEC strains belonging to O: 111 and O: 26 have been isolated from dairy cattle and man (Simmons, 1997, Desmarchelier, 1997, Karch *et al.*, 1997). Verotoxic *E. coli* strains of O: 111 and O: 26 serogroups caused symptoms of diarrhea, mucohaemorrhagic colitis, proctitis and bloody diarrhea in young calves (Schoonderwoerd *et al.*, 1988; Jnake *et al.*, 1990). Kapoor *et al.* (1995) found *E. coli* strains of serogroup O: 23, O: 25, O: 35, O: 66, O: 78, O: 86, O: 111, O: 125, O: 127 from diarrheic patients.

Earlier in many countries too verotoxic *E.coli* from faeces and raw bovine milk samples have been reported (Konowalchuk *et al.*, 1977, Chapman and Higgins, 1993., Abdul *et al.*, 1996). Number of samples of faeces positive for VTEC was 11 of 100 healthy cattle (2 calves, nine cows) and 1 of 100 diarrheoic calves. The prevalence of VTEC in healthy cattle in the present study is in agreement with the findings of Montenegro *et al.* (1990), who reported a similar isolation rate (10.8%) from healthy cattle indicating cattle as an important source of infection for human beings. In the present study, 1.0% faecal samples of diarrheic calves were positive for VTEC as compared to 11.0% of the samples of healthy cattle. Blanco *et al.* (1993) reported similarly a lower isolation rate of verotoxic *E. coli* from diarrheic calves (9.0%) than from healthy cattle (19%).

Isolation of verotoxic *E. coli* from healthy cattle might have serious health implications. Healthy animals living in symbiosis with human beings, manual handling of faeces of such animals and poor hygiene at the time of production of milk suggest that such cattle may pose a threat to human health.

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